Figure 1

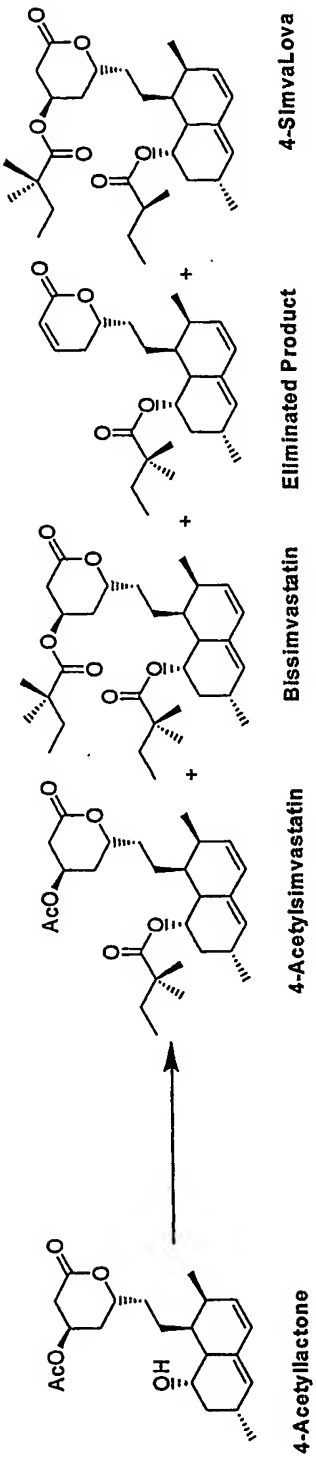

4-Acetyllactone
4-Acetylsimvastatin
Bissimvastatin
Eliminated Product
4-SimvaLova
AcO
OH
+
+
+

Figure 2

Table 3. Survey of conditions for the acylation of 4-acetyllactone

Run	BF_3 .OEt_2 mol%	DCM:MeCN	Time h	Mass[1] balance %	4-Ac Sim %	SM %	DiOAc %	4-Ac Lova %	Elimin %	4-Sim-Lova %	BisSim[3] %
1	8	1:1	0.5	94.8	90.8	1.2	2.8	1.1	0.5	2.7	0.9
2	8 (Old)	1:1	5.3	85.3	90.7	0.6	3.3	1.2	0.9	2.5	0.8
3	8	9:1	6.5	89.7	91.1	0.6	2.7	1.1	0.8	2.7	1.0
4	4	1:1	17.5	78.9	36.3	55.9	3.9	1.0	1.3	1.0	0.6
5	8	5:1	1.1	92.8	96.9	0.3	0.9	0.5	0.4		1.0
6	8 (Stock)	5:1	4.5	87.1	96.1	0.5	1.0	0.5	0.7		1.2

[1]Crude weight yield. [2]Lovastatin with 2,2-dimethylbutyrate at the 4-position. [3]2,2-Dimethylbutyrate at the 4,8-positions.

Figure 3

Table 4. Purification of 4-Acetylsimvastatin by precipitation from MeOH

	DCE:MeCN	Wt yield %	4-AcSim %	SM %	DiOAc %	4-Ac Lova %	Elimin %	BisSim %
Crude product	5:1	112[1]	96.4	1.0	0.9	0.3	0.7	0.7
After precipitation		93	98.5	0.2	0.9	0.2	0.2	

[1] Contaminated with 2,2-dimethylbutyic acid

Figure 4

Table 8. Isolation of Simvastatin

Run	Scale g	Batch	Theoret Yield g	Isolated Yield g	Yield %	Acid %	Simva %	4-Acsim %	Elimin %	Lova %
1	50	Pooled: various	45.4	33.8	74.4	0.2	96.7	1.7	1.2	0.2
2	45	Pooled same batch	40.9	27.7	67.7	0.3	97.8	1.3	0.3	0.4
3	25	Pooled Same batch	22.7	17.2	75.7	0.3	97.7	1.3	0.3	0.4
4	96.6	Single batch	87.8	73.4	83.6	0.7	97.5	1.0	0.6	0.2

Figure 5

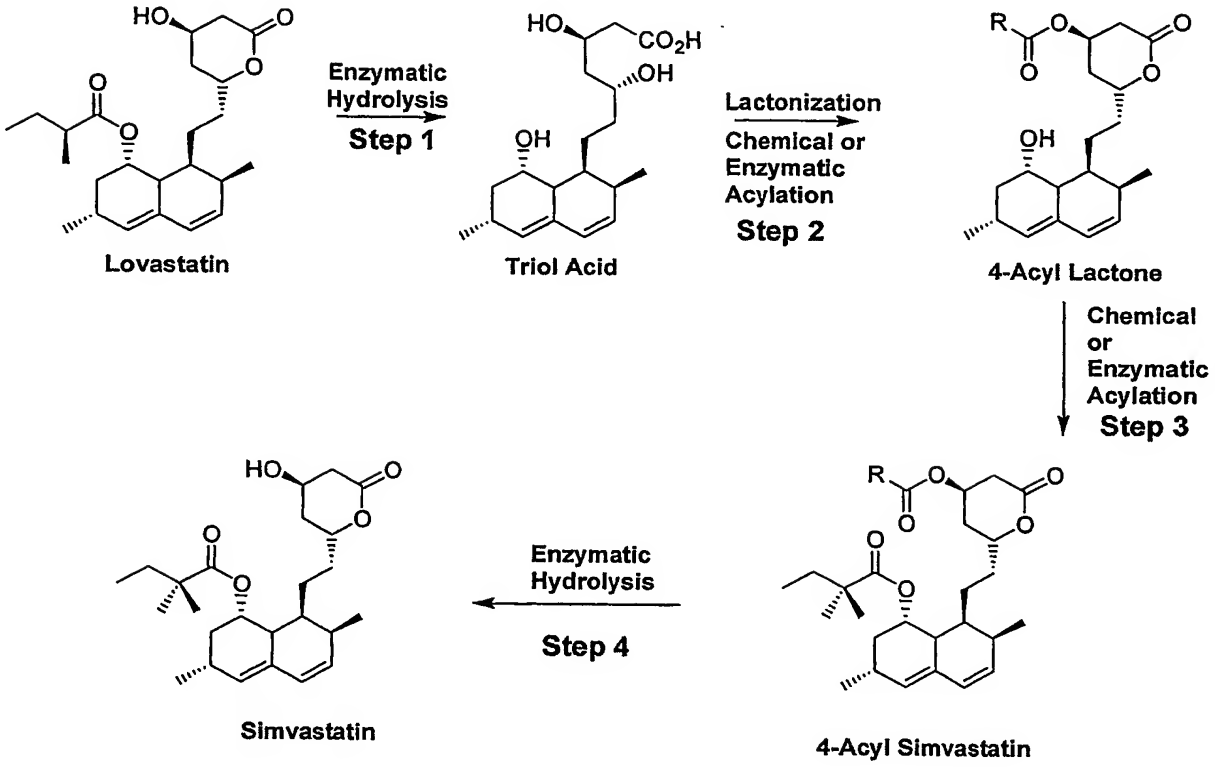

Lovastatin
Enzymatic Hydrolysis
Step 1
Triol Acid
Lactonization
Chemical or Enzymatic Acylation
Step 2
4-Acyl Lactone
Chemical or Enzymatic Acylation
Step 3
4-Acyl Simvastatin
Enzymatic Hydrolysis
Step 4
Simvastatin

Figure 6

Figure 6A

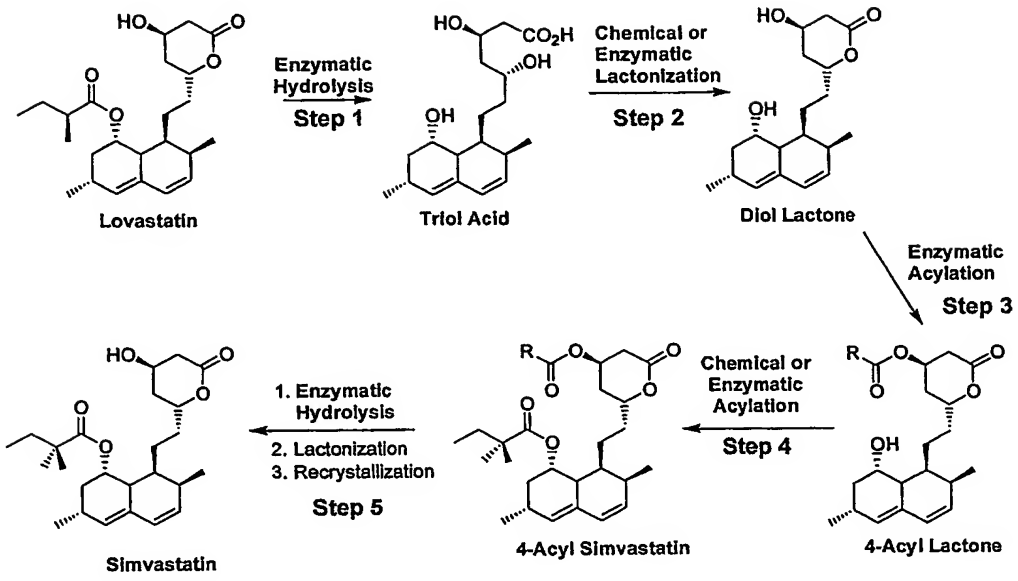


Figure 6B

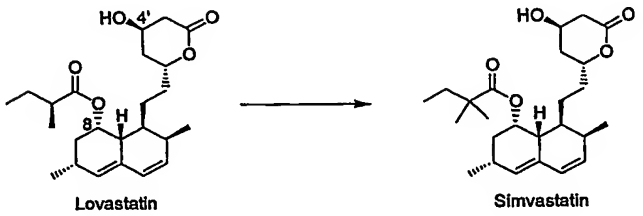


Figure 7

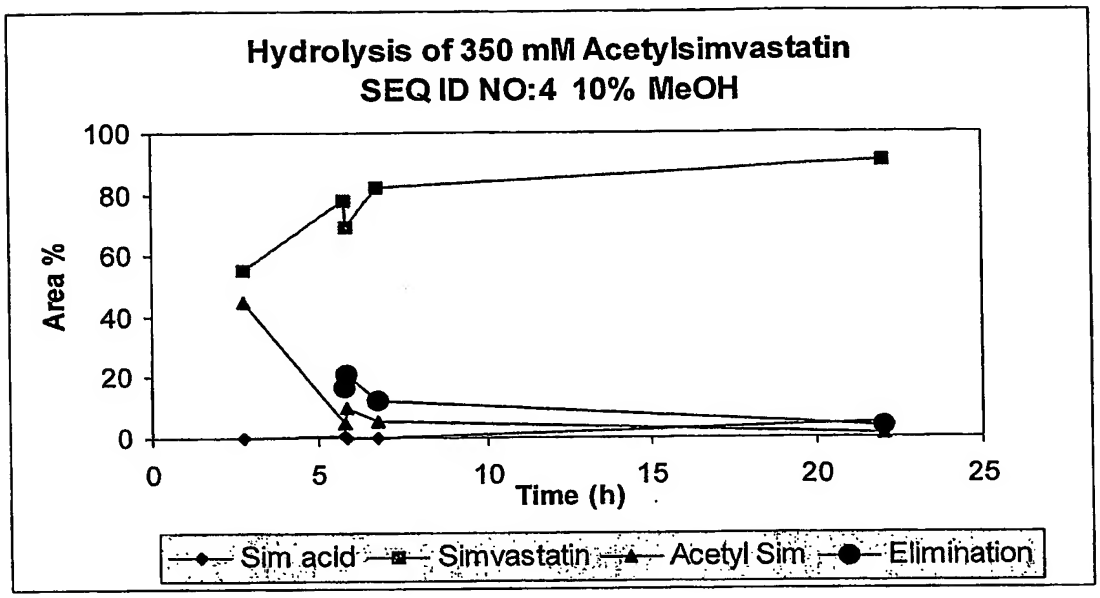

Hydrolysis of 350 mM Acetylsimvastatin
SEQ ID NO:4 10% MeOH
Area %
100
80
60
40
20
0
0
5
10
15
20
25
Time (h)
Sim acid
Simvastatin
Acetyl Sim
Elimination

Figure 8

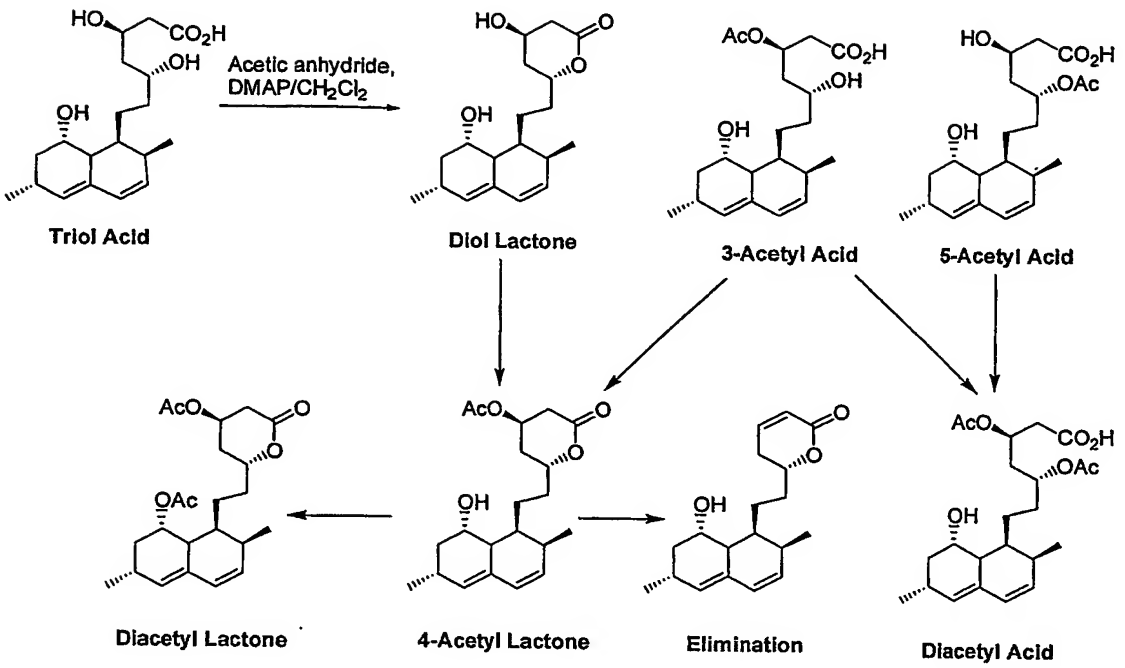
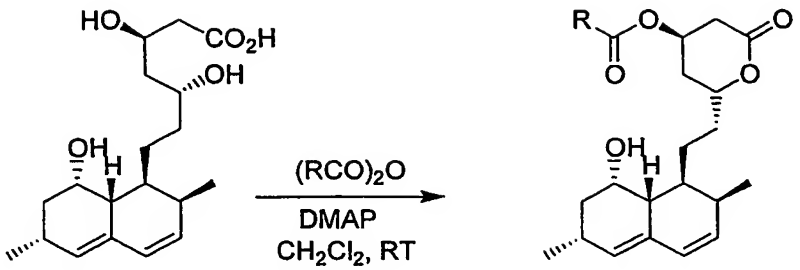

HO
CO2H
OH
OH
Acetic anhydride,
DMAP/CH2Cl2
Triol Acid
Diol Lactone
AcO
3-Acetyl Acid
5-Acetyl Acid
OAc
Diacetyl Lactone
4-Acetyl Lactone
Elimination
Diacetyl Acid

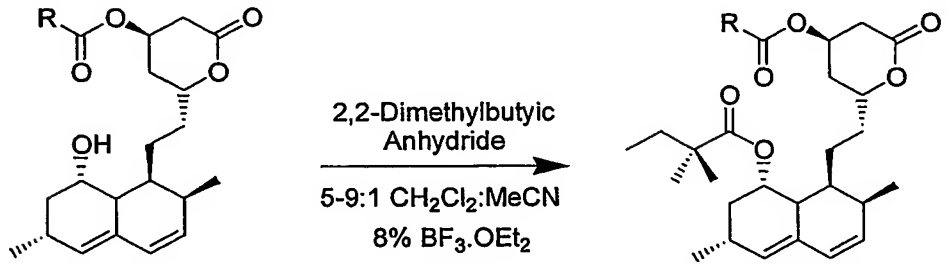
Figure 9

Figure 9A



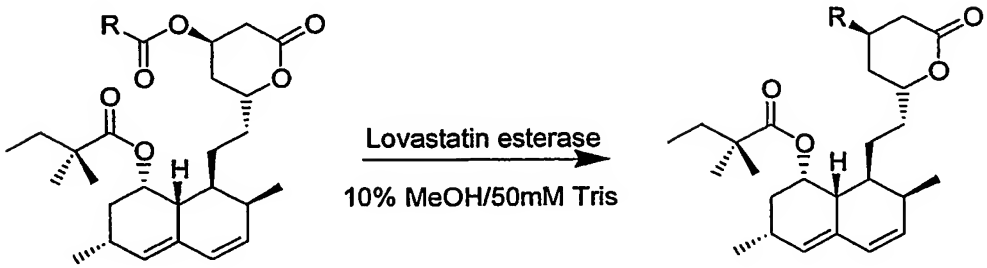
Triol Acid **4-Acyl Lactone**

Figure 9B



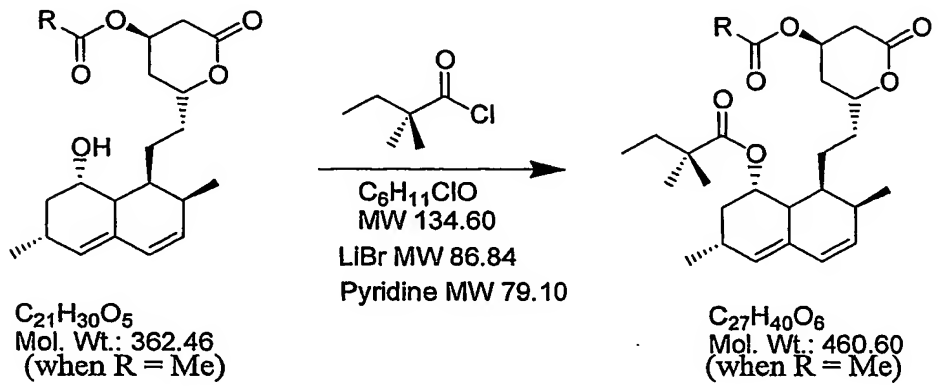
4-Acyl Lactone **4-Acyl Simvastatin**

Figure 9C



4-Acyl Simvastatin **Simvastatin**

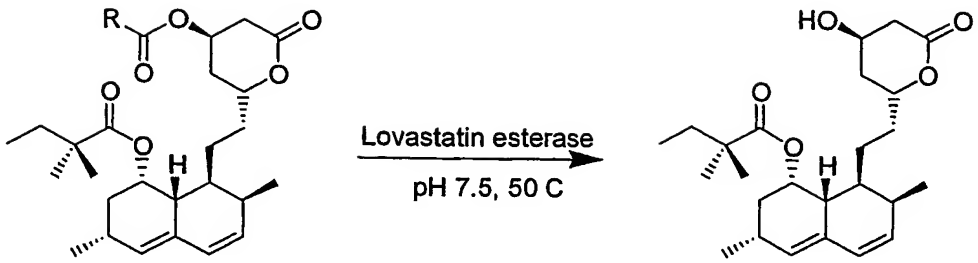
Figure 10



$C_{21}H_{30}O_5$
Mol. Wt.: 362.46
(when R = Me)

$C_{27}H_{40}O_6$
Mol. Wt.: 460.60
(when R = Me)

Figure 11



4-Acyl Simvastatin **Simvastatin**

Figure 12

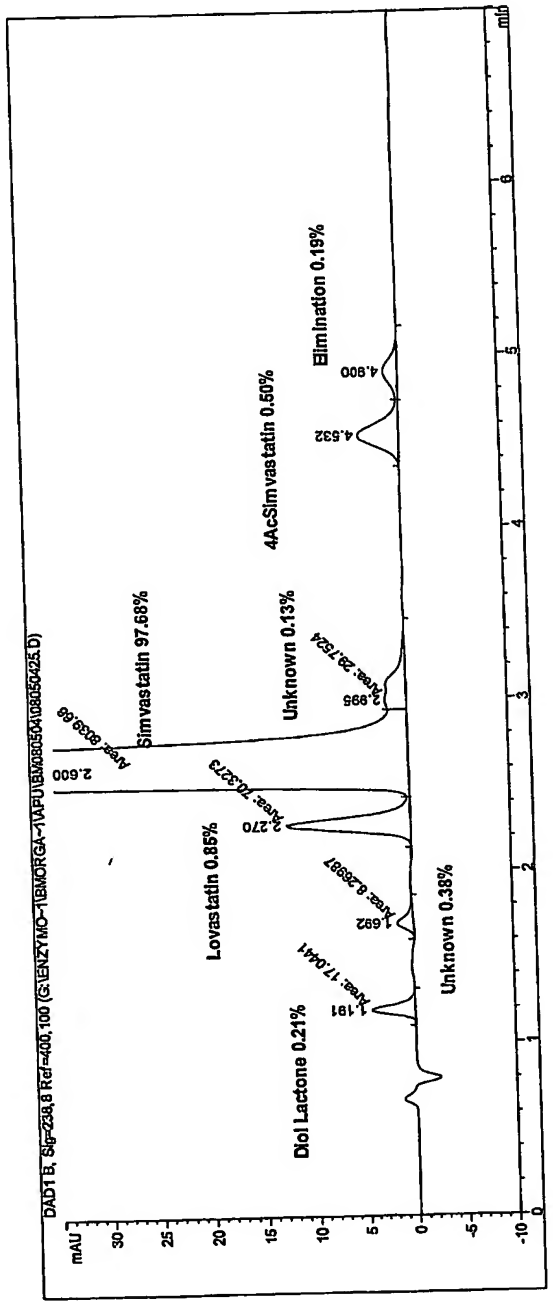

DAD1 B, Sig=238,8 Ref=400,100 (G:\ENZYMO~1\BMORGA~1\APU\BM080504\080504025.D)
mAU
min
Simvastatin 97.68%
Unknown 0.13%
4AcSimvastatin 0.50%
Elimination 0.19%
Lovastatin 0.85%
Diol Lactone 0.21%
Unknown 0.38%
2.600
2.995
4.532
4.800
2.270
1.692
1.181

Figure 13

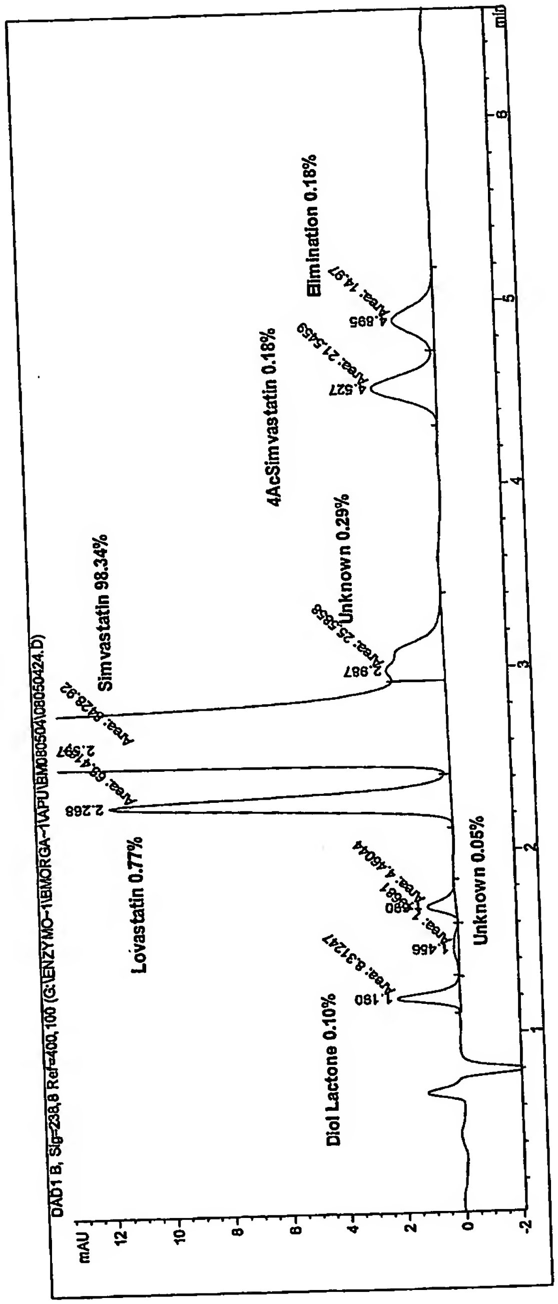

DAD1 B, Sig=238,8 Ref=400,100 (G:\ENZYMO~1\EMORGA~1\APU\EM080504\08050424.D)
mAU
min
Diol Lactone 0.10%
Lovastatin 0.77%
Simvastatin 98.34%
Unknown 0.05%
Unknown 0.29%
4AcSimvastatin 0.18%
Elimination 0.18%
2.268
2.597
2.887
4.527
4.895
Area: 8428.92
Area: 25.5858
Area: 21.5459
Area: 14.97

Figure 14

Run	Starting Material	Diol lactone (%)	Diacetyl lactone (%)	Elimination or Diacetyl Acid (%)	4-Acetyl Lactone (%)	Yield %	Mass Balance (%)
1	Triol acid	0.5	1.7	5.1	91.1	87.4	94.2
2	Triol acid	0.5	1.7	6.4	91.0	83.2	93.0
3	Triol acid	2.3	1.3	8.1	88.3	88.8	100.9
4	Triol acid	2.4	1.3	8.1	88.2	90.9	103.1

Conditions: DMAP 15 mol%; Anhydride: 3 eq; 0°-RT, CH_2Cl_2

Figure 15

Figure 15A

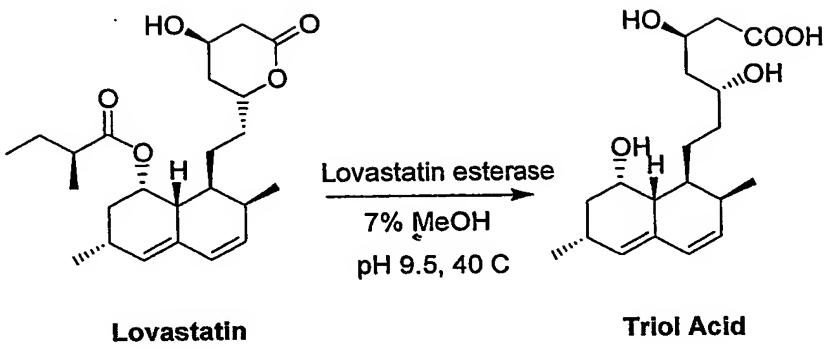


Figure 15B

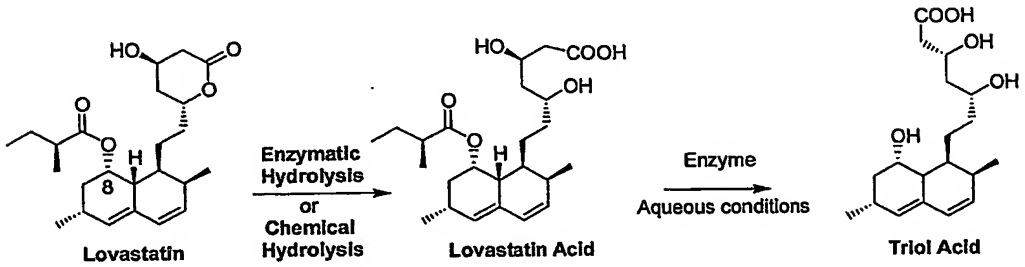


Figure 16

Figure 16A

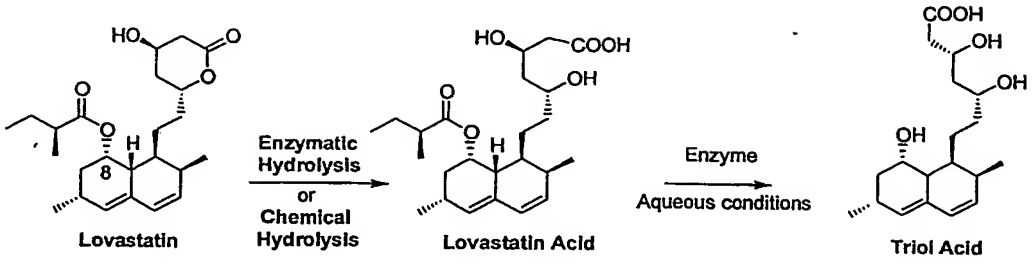


Figure 16B

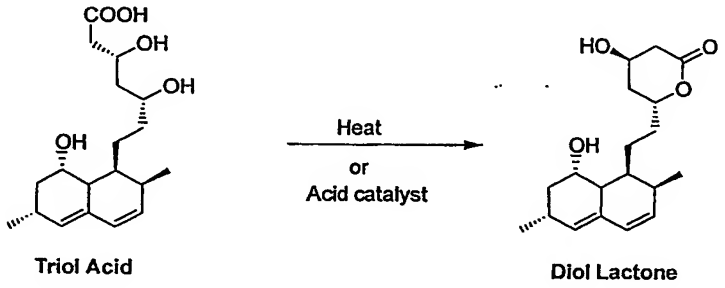


Figure 16C

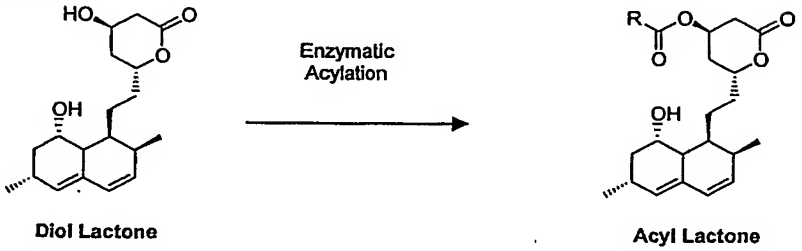


Figure 16D

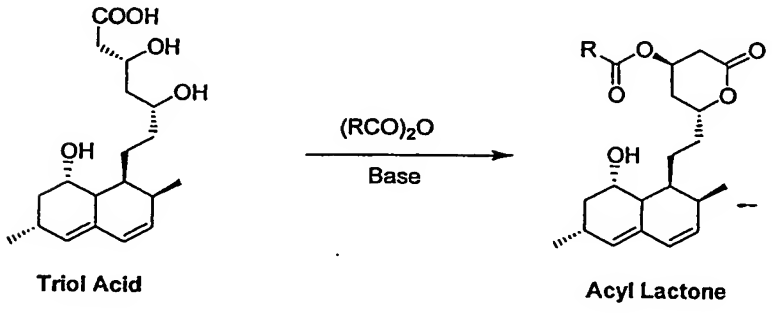


Figure 16

Figure 16E

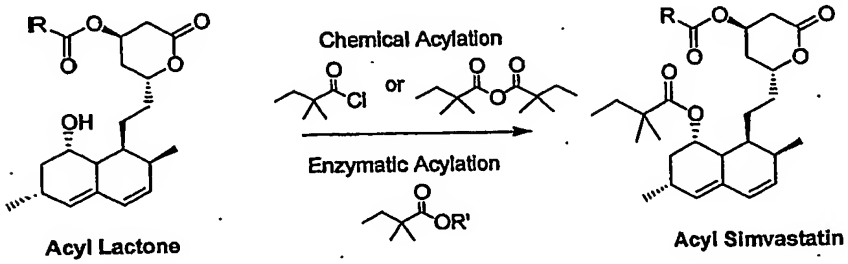


Figure 16F

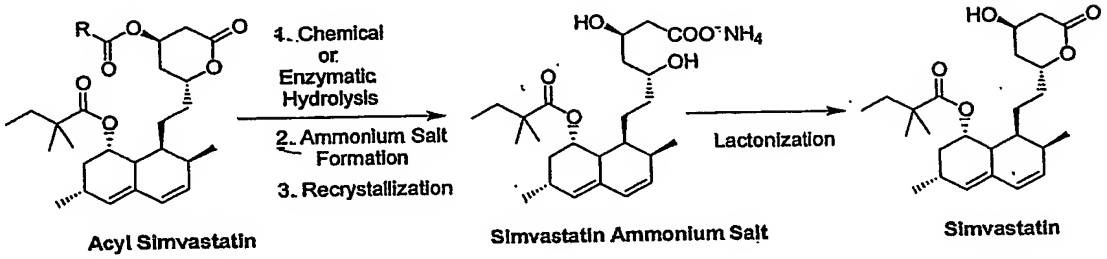


Figure 17

Figure 17A

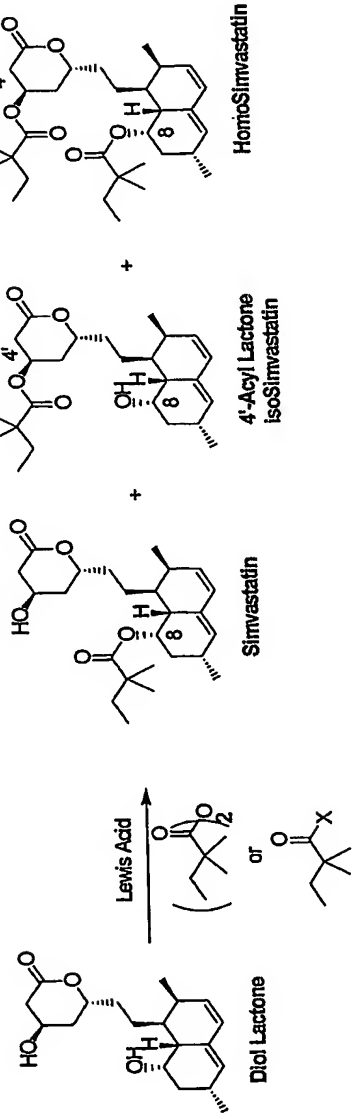


Figure 17B

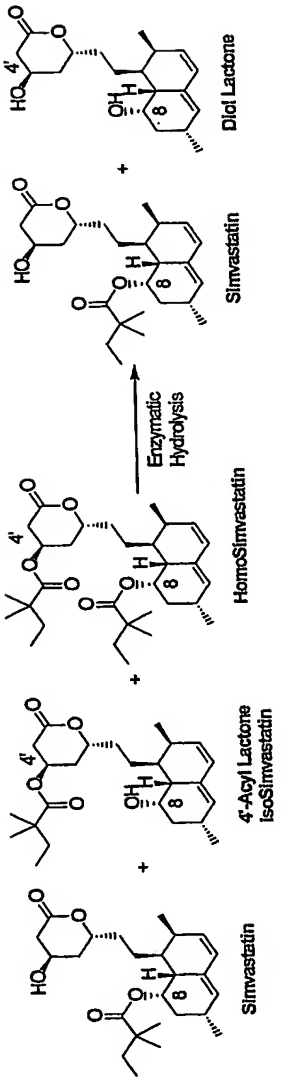
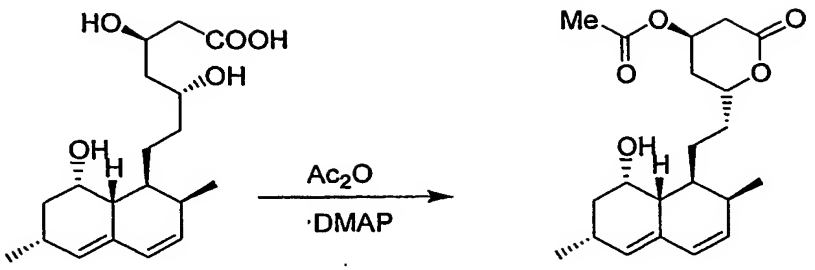


Figure 18

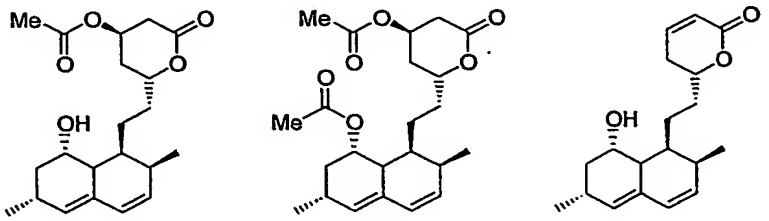
Figure 18A



$C_{19}H_{30}O_5$
Mol. Wt.: 338.44

$C_{21}H_{30}O_5$
Mol. Wt.: 362.46

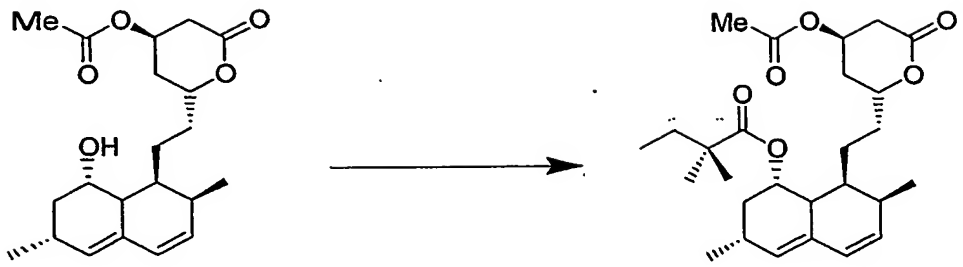
Figure 18B



4-Acetyl Lactone **Diacetate** **Elimination Product**

Figure 18

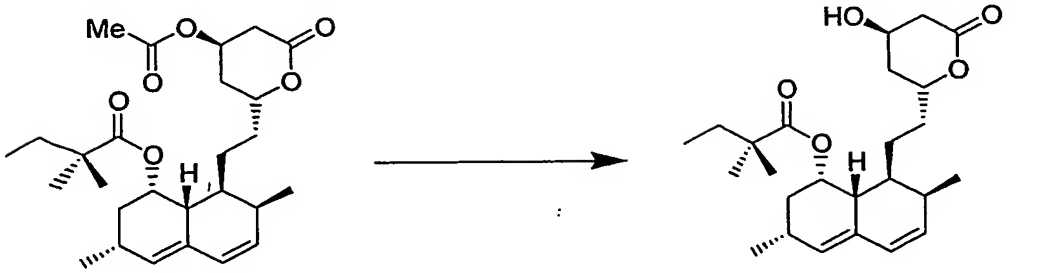
Figure 18C



$C_{21}H_{30}O_5$
Mol. Wt.: 362.46

$C_{27}H_{40}O_6$
Mol. Wt.: 460.60

Figure 18D

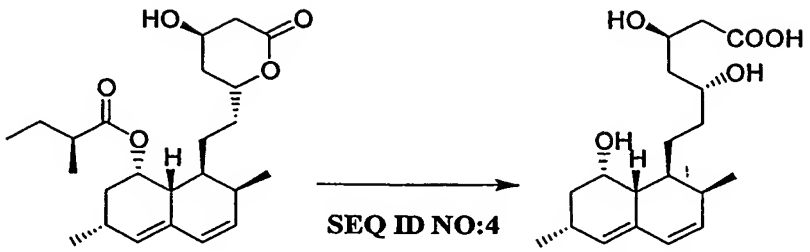


$C_{27}H_{40}O_6$
Mol. Wt.: 460.60

$C_{25}H_{38}O_5$
Mol. Wt.: 418.57

Figure 18

Figure 18E



$C_{24}H_{36}O_5$
Mol. Wt.: 404.54

$C_{19}H_{30}O_5$
Mol. Wt.: 338.44

Figure 19

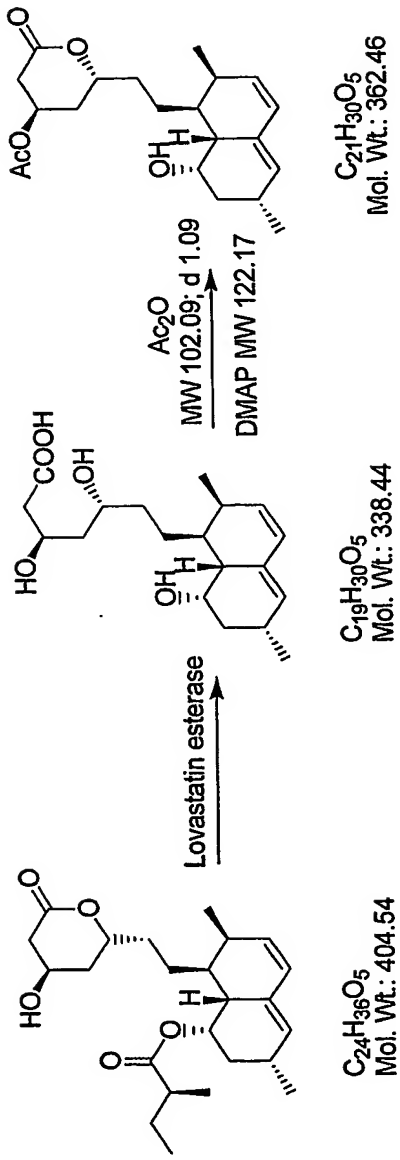

Lovastatin esterase
$C_{24}H_{36}O_5$
Mol. Wt.: 404.54
$C_{19}H_{30}O_5$
Mol. Wt.: 338.44
Ac_2O
MW 102.09; d 1.09
DMAP MW 122.17
$C_{21}H_{30}O_5$
Mol. Wt.: 362.46

Figure 20

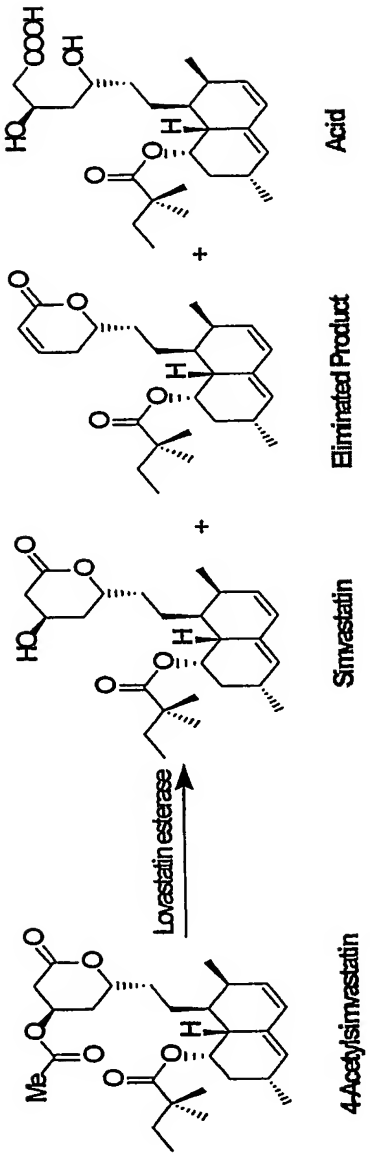

Lovastatin esterase
4Acetylsimvastatin
Simvastatin
Eliminated Product
Acid

Figure 21

	Triol Acid	Diol Lactone	1.66	Lovast atin	Simvas tatin	2.85	AcLov	AcSimv	Elimi	HPLC Assay %	CHN
M-31		0.11		0.34	99.5						√
2645-82/83	0.47	0.15	0.08	0.31	97.0	0.47		0.92	0.19	84.5	X
2645-84		0.96	0.73	0.48	95.2	0.39		0.93	1.35	86.3	√
ML 38 g		0.43	0.62	0.45	96.8	0.55	0.25	0.52	0.36	93.5	n/d

Figure 22

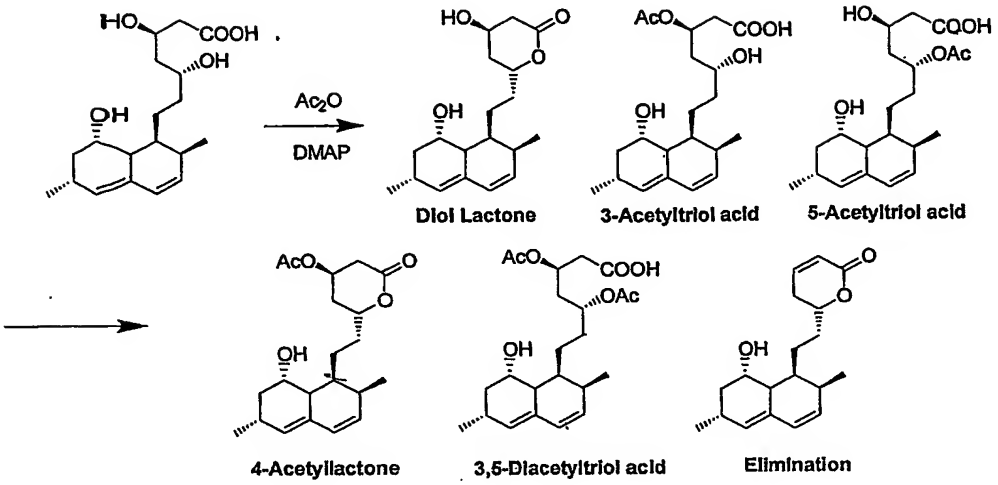

HO
COOH
OH
OH
Ac2O
DMAP
Diol Lactone
AcO
3-Acetyltriol acid
5-Acetyltriol acid
4-Acetyllactone
3,5-Diacetyltriol acid
Elimination

Figure 23

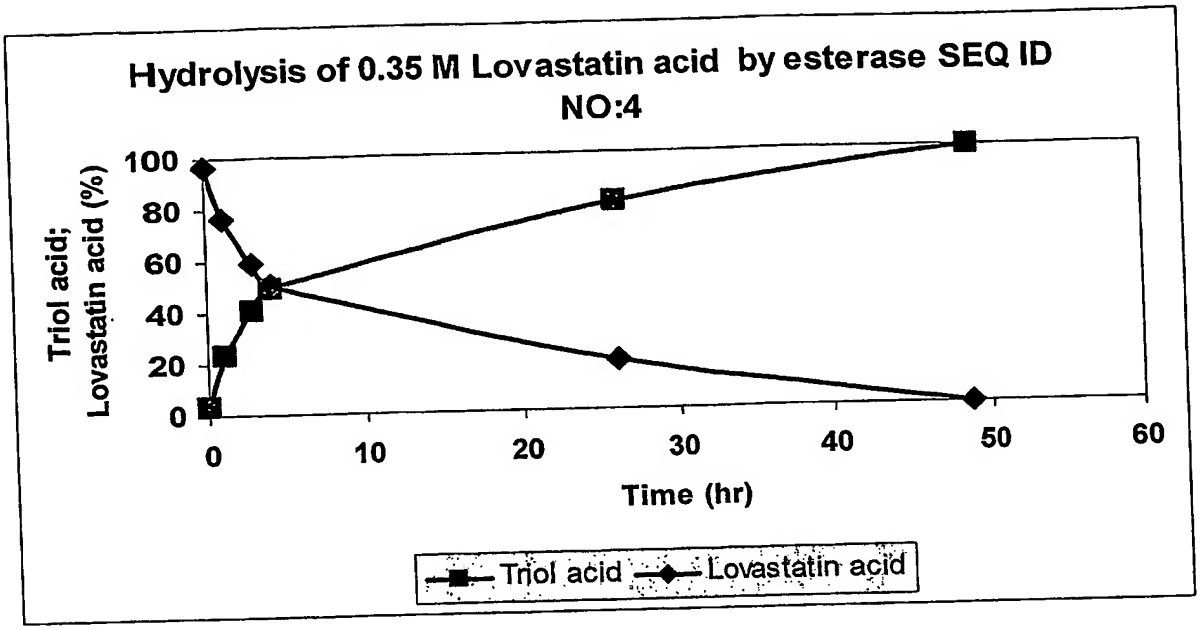
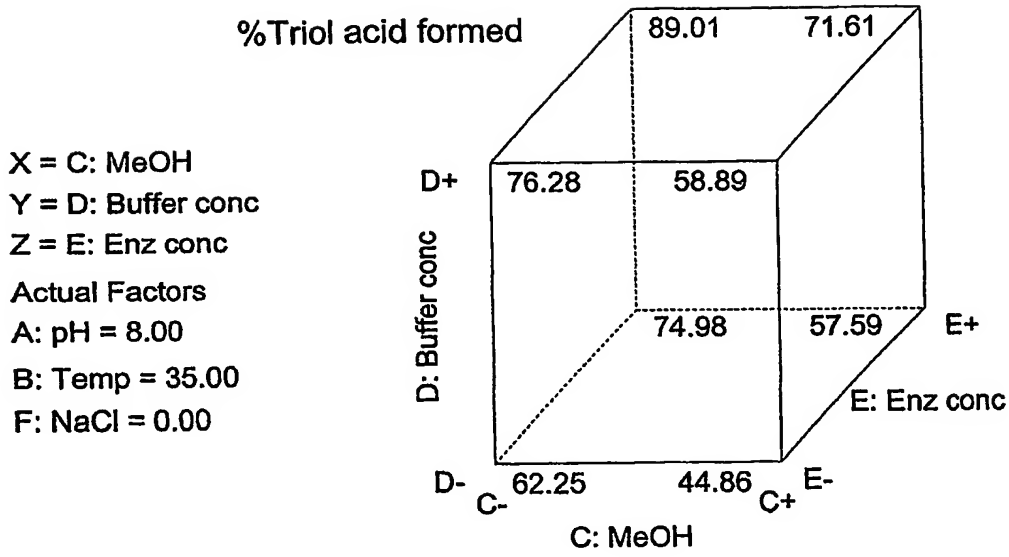

Hydrolysis of 0.35 M Lovastatin acid by esterase SEQ ID NO:4
Triol acid; Lovastatin acid (%)
100
80
60
40
20
0
0
10
20
30
40
50
60
Time (hr)
Triol acid
Lovastatin acid

Figure 24

Run no	Block	Parameter levels												Responses	
		pH		Temp oC		MeOH %		Tris-HCl mM		Enzyme mUMB U [1]		NaCl mM		Rate [2]	Conversion [3]
1	Block 1	+1	9.5	-1	35	+1	20	-1	25	-1	8.4	+1	500	3.69	45.07
2	Block 1	+1	9.5	+1	45	+1	20	-1	25	+1	20	-1	0	2.15	41.35
3	Block 1	-1	8	-1	35	+1	20	+1	200	+1	20	+1	500	4.62	52.33
4	Block 1	-1	8	+1	45	+1	20	+1	200	-1	8.4	-1	0	5.43	54.22
5	Block 1	+1	9.5	-1	35	-1	0	+1	200	+1	20	-1	0	14.84	94.86
6	Block 1	-1	8	+1	45	-1	0	-1	25	+1	20	+1	500	13.89	65.62
7	Block 1	-1	8	-1	35	-1	0	-1	25	-1	8.4	-1	0	5.43	63.11
8	Block 1	+1	9.5	+1	45	-1	0	+1	200	-1	8.4	+1	500	13.27	54.38
9	Block 2	+1	9.5	-1	35	-1	0	-1	25	+1	20	+1	500	19.48	60.37
10	Block 2	-1	8	+1	45	+1	20	-1	25	-1	8.4	+1	500	4.90	32.87
11	Block 2	-1	8	-1	35	-1	0	+1	200	-1	8.4	+1	500	6.86	59.52
12	Block 2	-1	8	-1	35	+1	20	-1	25	+1	20	-1	0	6.46	59.84
13	Block 2	-1	8	+1	45	-1	0	+1	200	+1	20	-1	0	20.75	96.06
14	Block 2	+1	9.5	+1	45	+1	20	+1	200	+1	20	+1	500	11.22	66.58
15	Block 2	+1	9.5	+1	45	-1	0	-1	25	-1	8.4	-1	0	12.38	61.76
16	Block 2	+1	9.5	-1	35	+1	20	+1	200	-1	8.4	-1	0	4.66	64.27

Figure 25

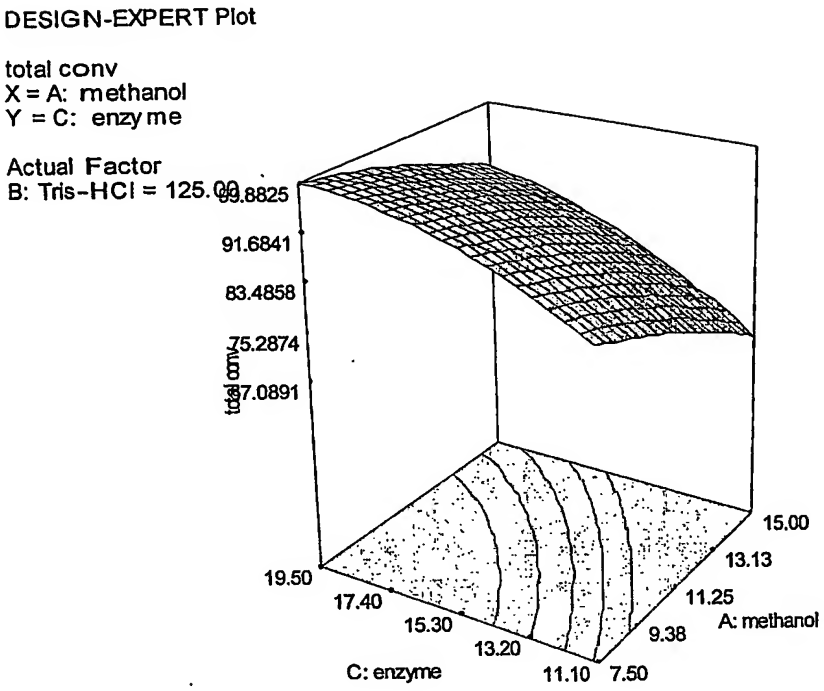


Parameter	Level	Effect on triol acid production
MeOH	High	Negative
Tris-HCl conc.	High	Positive
Enzyme conc.	High	Positive
NaCl conc.	High	Negative
pH		No significant effect
Temperature		No significant effect

Figure 26

Run no	Block	Parameter levels						Responses	
		Methanol		Tris-HCl		Enzyme		Rate [2]	Conversion [3]
			%		mM		mUMB U [1]		
1	Block 1	-1	7.50	-1	50	-1	11.1	6.58	85.00
2	Block 1	+1	15.00	+1	200	-1	11.1	4.50	62.52
3	Block 1	0	11.25	0	125	0	15.3	7.71	88.23
4	Block 1	+1	15.00	-1	50	+1	19.5	7.55	87.55
5	Block 1	-1	7.50	+1	200	+1	19.5	11.30	98.23
6	Block 1	+1	11.25	0	125	0	15.3	7.40	88.24
7	Block 2	-1	7.50	-1	50	+1	19.5	11.88	99.60
8	Block 2	+1	11.25	0	125	0	15.3	7.53	88.85
9	Block 2	-1	7.50	+1	200	-1	11.1	6.84	84.19
10	Block 2	0	15.00	+1	200	+1	19.5	7.73	88.84
11	Block 2	0	15.00	-1	50	-1	11.1	5.33	73.40
12	Block 2	+1	11.25	0	125	0	15.3	8.20	93.89
13	Block 3	+1	11.25	0	125	0	15.3	7.18	87.79
14	Block 3	+1	11.25	-1.4	20	0	15.3	7.30	89.24
15	Block 3	+1	11.25	1.4	230	0	15.3	7.73	90.75
16	Block 3	+1.4	16.50	0	125	0	15.3	5.64	76.20
17	Block 3	+1	11.25	0	125	1.4	21.18	10.43	98.03
18	Block 3	+1	11.25	0	125	0	15.3	7.35	89.94
19	Block 3	-1.4	6.00	0	125	0	15.3	10.33	98.20
20	Block 3	+1	11.25	0	125	-1.4	9.42	4.53	68.85

Figure 27


DESIGN-EXPERT Plot
total conv
X = A: methanol
Y = C: enzyme
Actual Factor
B: Tris-HCl = 125.00
99.8825
91.6841
83.4858
75.2874
67.0891
total conv
19.50
17.40
15.30
13.20
11.10
C: enzyme
7.50
9.38
11.25
13.13
15.00
A: methanol

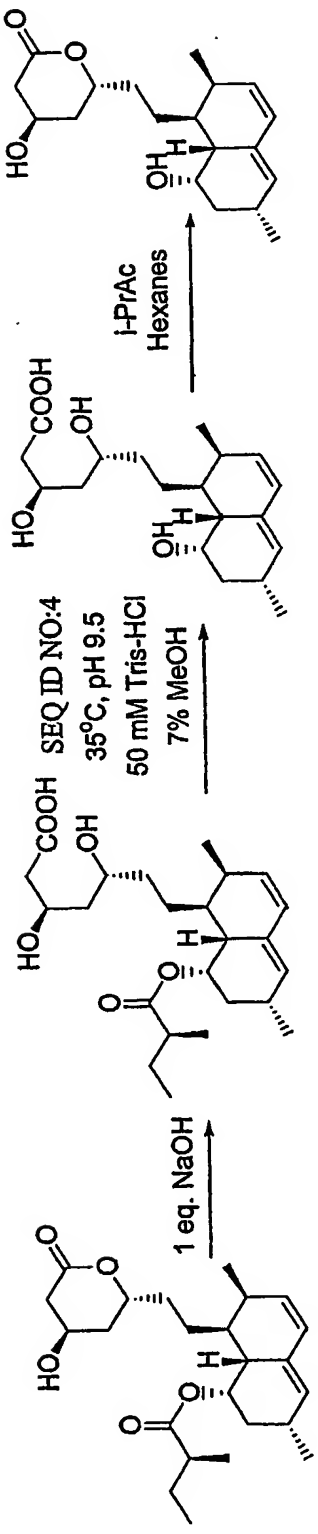

1 eq. NaOH
SEQ ID NO:4
35°C, pH 9.5
50 mM Tris-HCl
7% MeOH
i-PrAc
Hexanes
COOH
OH
HO
H
O

Figure 28

Figure 29

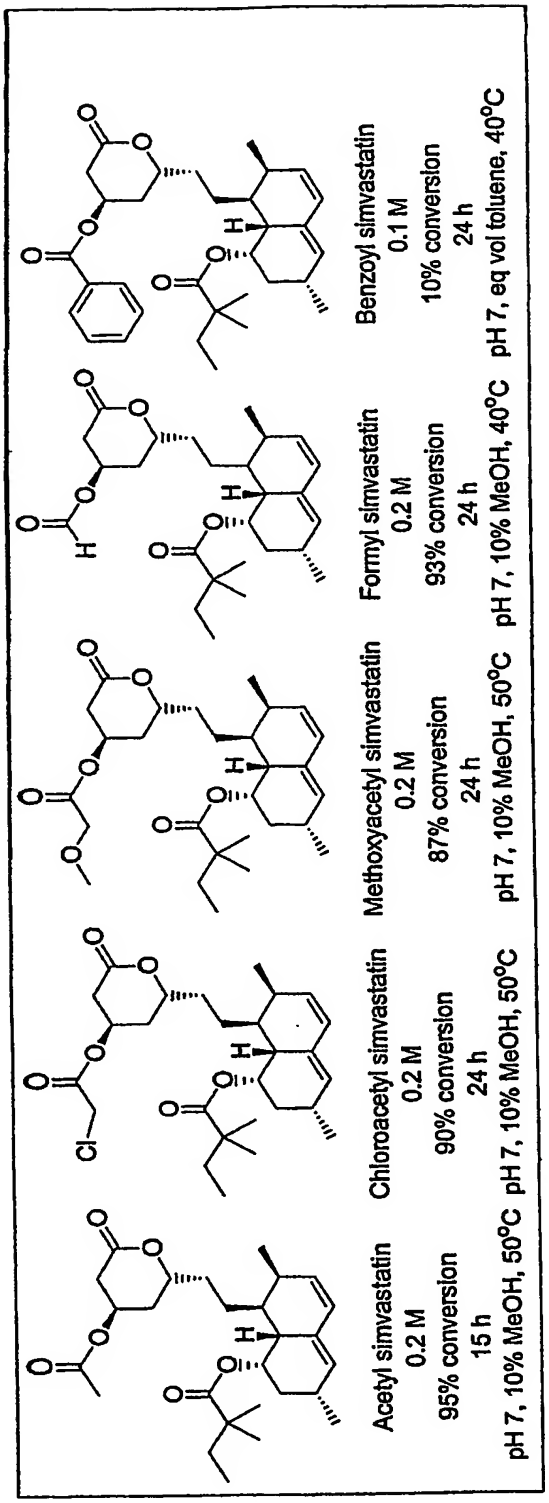

Acetyl simvastatin
0.2 M
95% conversion
15 h
pH 7, 10% MeOH, 50°C
Chloroacetyl simvastatin
0.2 M
90% conversion
24 h
pH 7, 10% MeOH, 50°C
Methoxyacetyl simvastatin
0.2 M
87% conversion
24 h
pH 7, 10% MeOH, 50°C
Formyl simvastatin
0.2 M
93% conversion
24 h
pH 7, 10% MeOH, 40°C
Benzoyl simvastatin
0.1 M
10% conversion
24 h
pH 7, eq vol toluene, 40°C

Figure 30

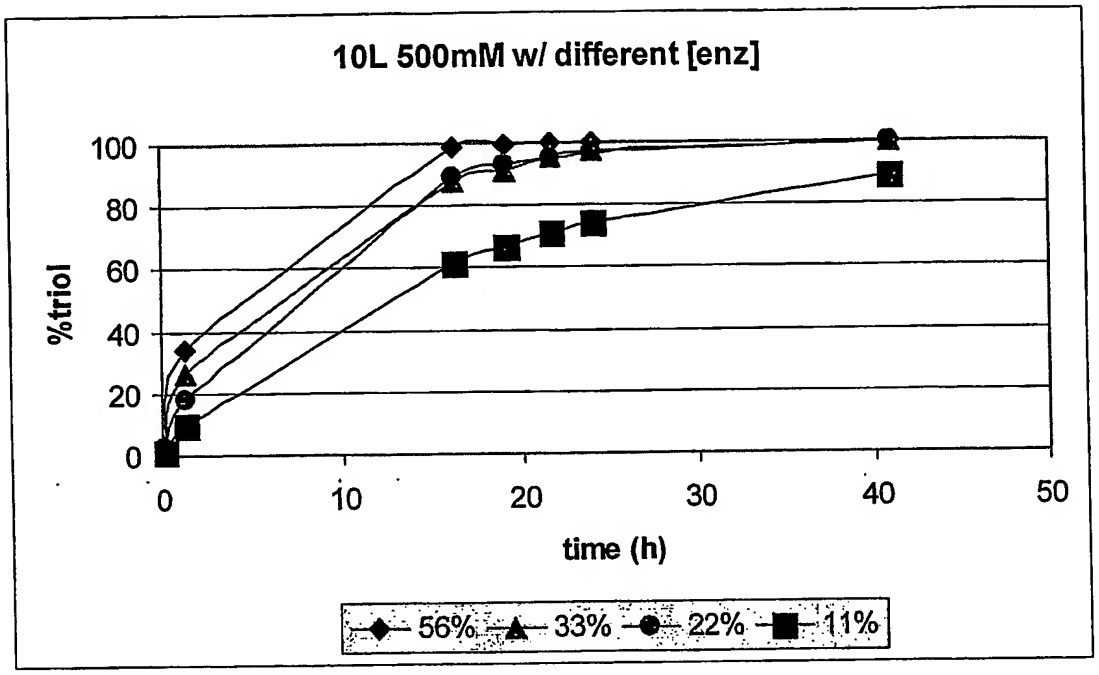
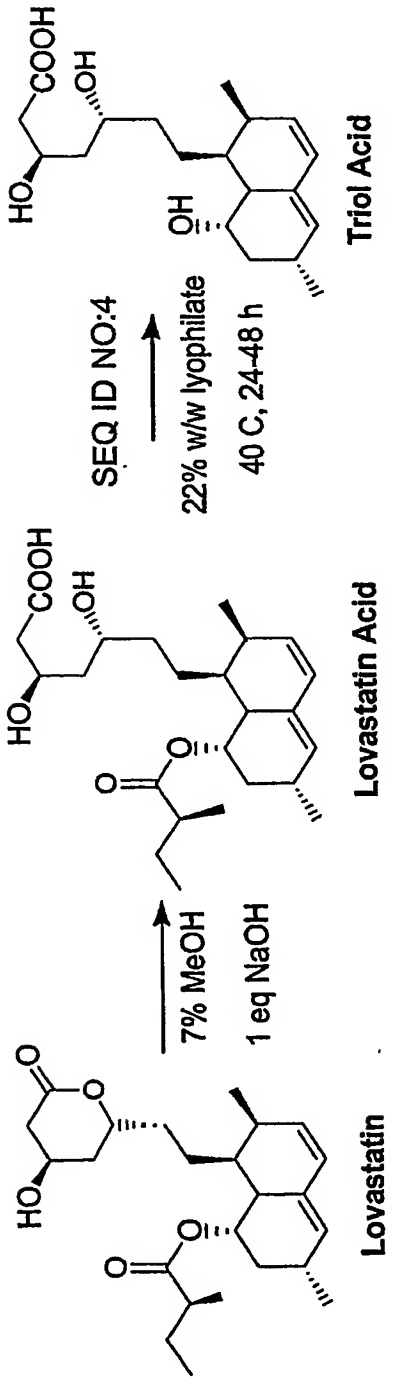

10L 500mM w/ different [enz]
%triol
100
80
60
40
20
0
0
10
20
30
40
50
time (h)
56%
33%
22%
11%

Figure 31



Substrate g	Lyophilate g	Time h	Conversion %
50	11.3	28	99
100	22.6	28	97.5
		42	99.4
100	22.6	28	97.8
		44	>99

Substrate 500 mM; 7% MeOH; 40 C

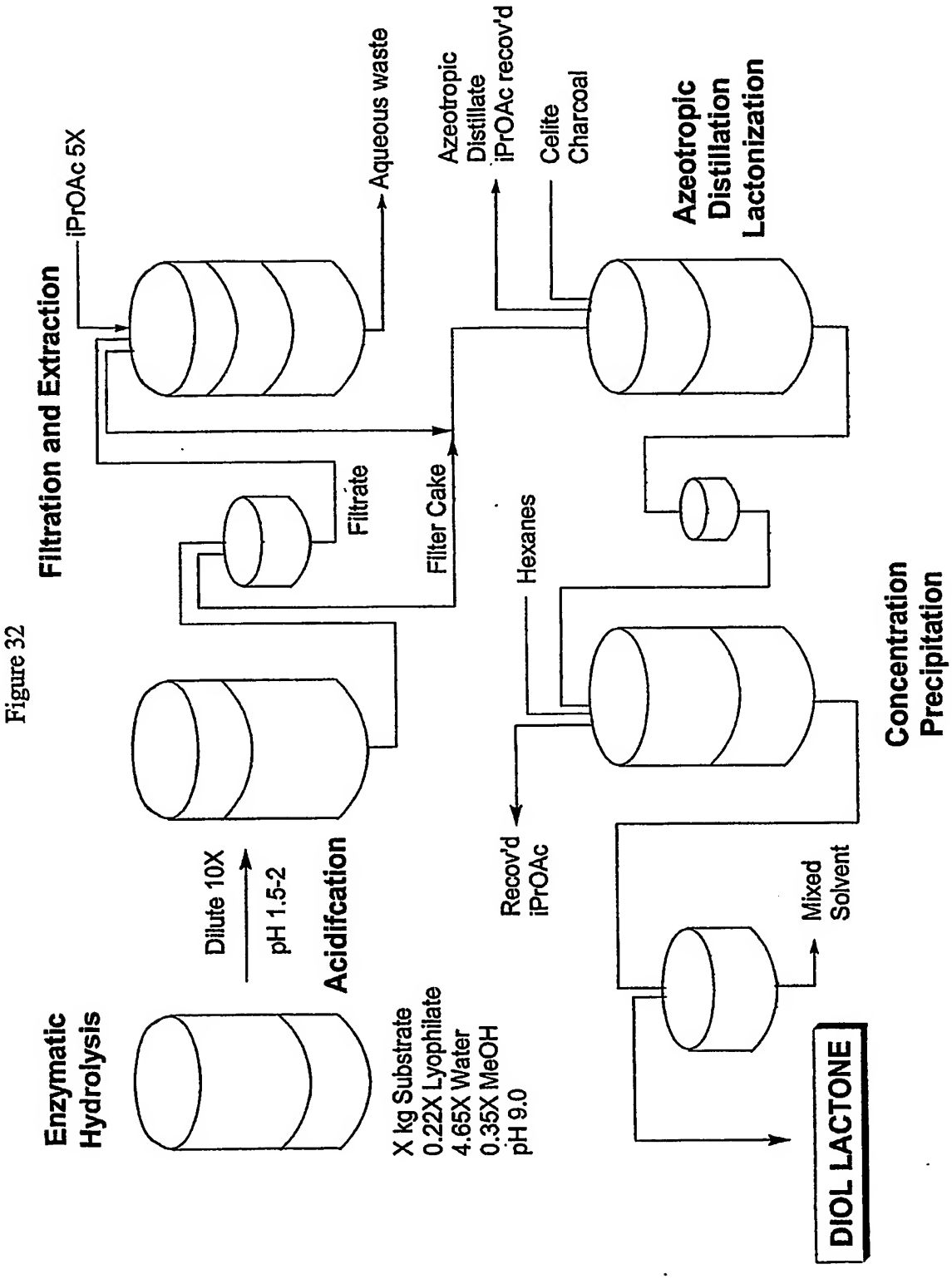
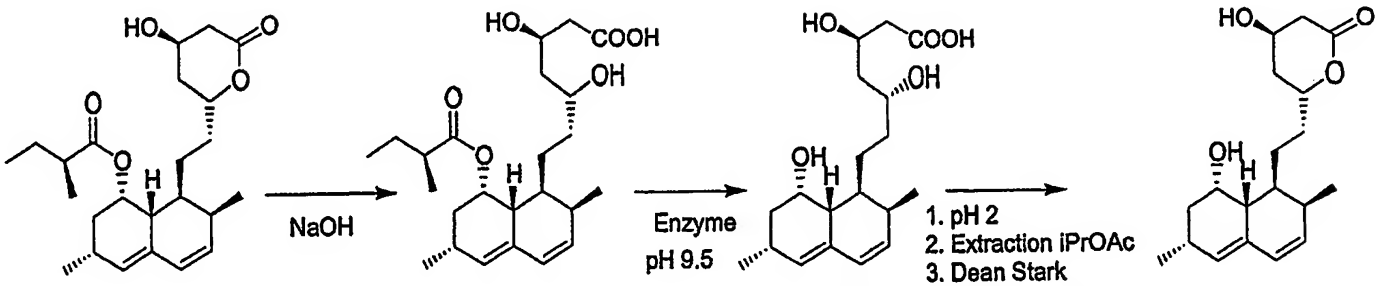

Enzymatic Hydrolysis
X kg Substrate
0.22X Lyophilate
4.65X Water
0.35X MeOH
pH 9.0
Acidifcation
Dilute 10X
pH 1.5-2
Filtration and Extraction
iPrOAc 5X
Aqueous waste
Filtrate
Filter Cake
Azeotropic Distillate
iPrOAc recov'd
Celite
Charcoal
Azeotropic Distillation Lactonization
Hexanes
Recov'd iPrOAc
Mixed Solvent
Concentration Precipitation
DIOL LACTONE

Figure 32

Figure 33



MW 404.54 **Lovastatin**

MW 422.55 **Lovastatin Acid**

MW 338.44 **Triol Acid**

MW 320.42 **Diol Lactone**

Reactions: Scale/mM	Workup	Theoretical Yield g	Product g	Yield %	
350 mM pooled	Continuous Liquid/Liquid Dean Stark	5	4.19	83.8	90% accounted
12 g 500 mM	Centrifugation MeOH	9.58	7.85 Two crops	81.9	91.9% accounted
10.8 g 500 mM	Warm extraction Centrifugation	8.62	6.63	76.9	85% accounted
50 g 500 mM	Centrifugation	39.6	39.2 evaporated	99	0.6 g in washes
100 g 500 mM	Filtration	79.2	61.6	77.8	90.8% accounted

Figure 34

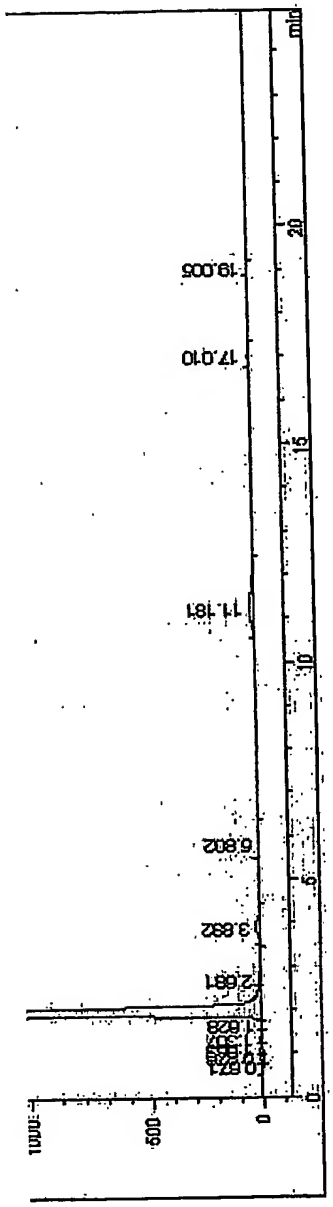


Figure 34A

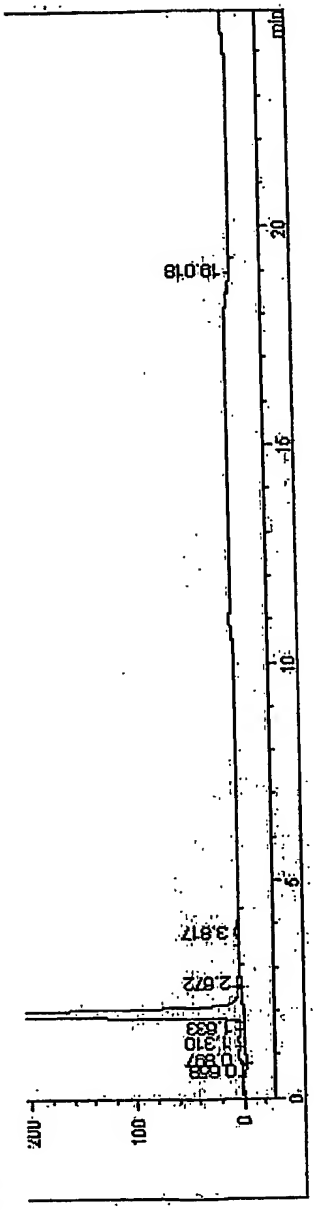


Figure 34B

Figure 35

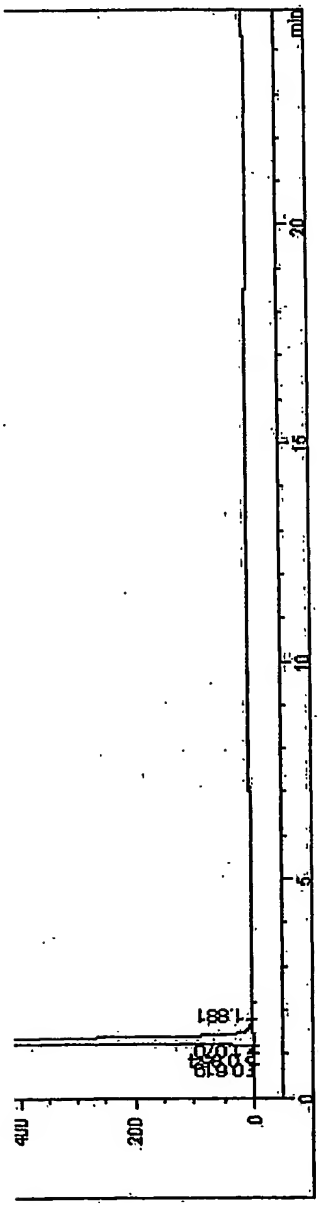


Figure 35A

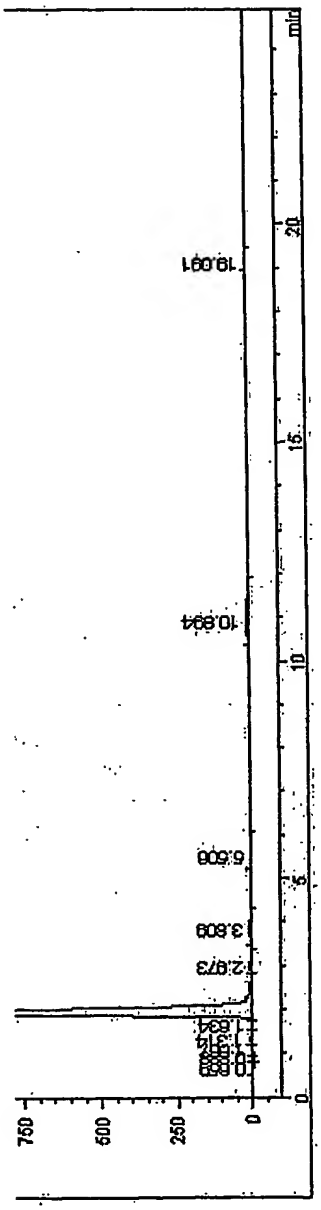
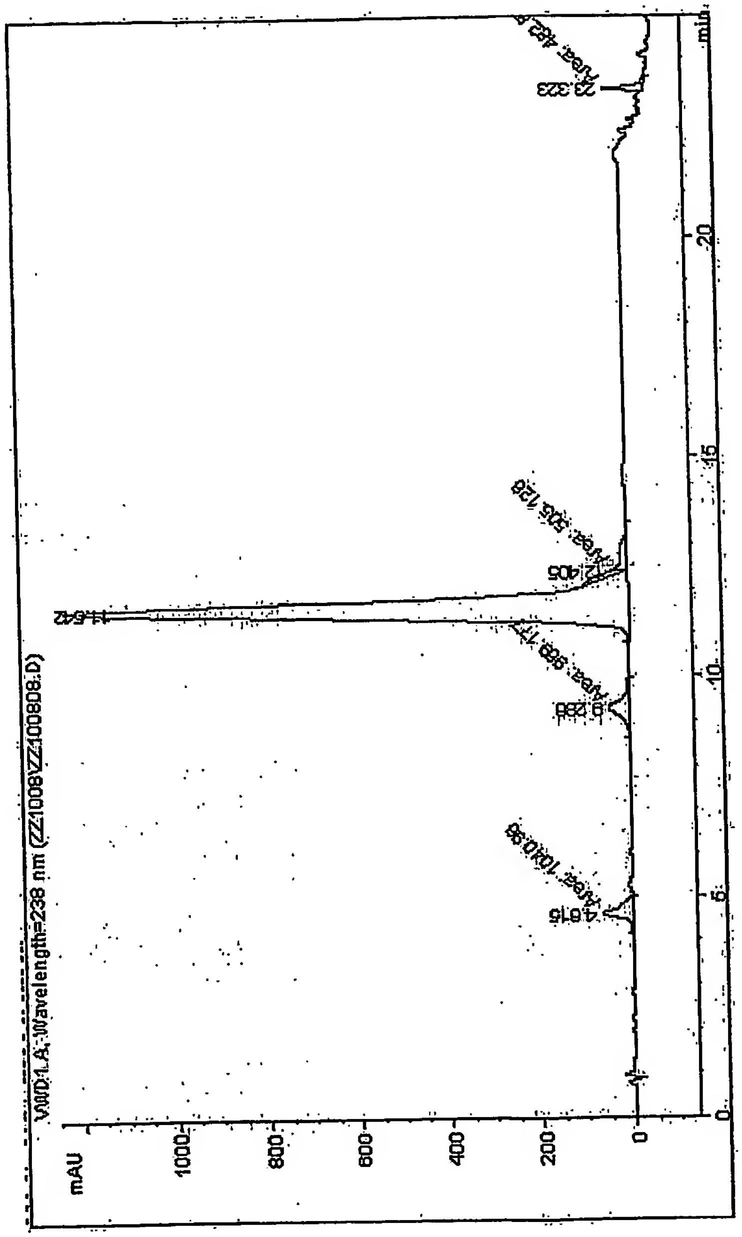


Figure 35B

Figure 36


mAU
1000
800
600
400
200
0
0
5
10
15
20
min
4.615
9.286
11.642
12.405
23.323

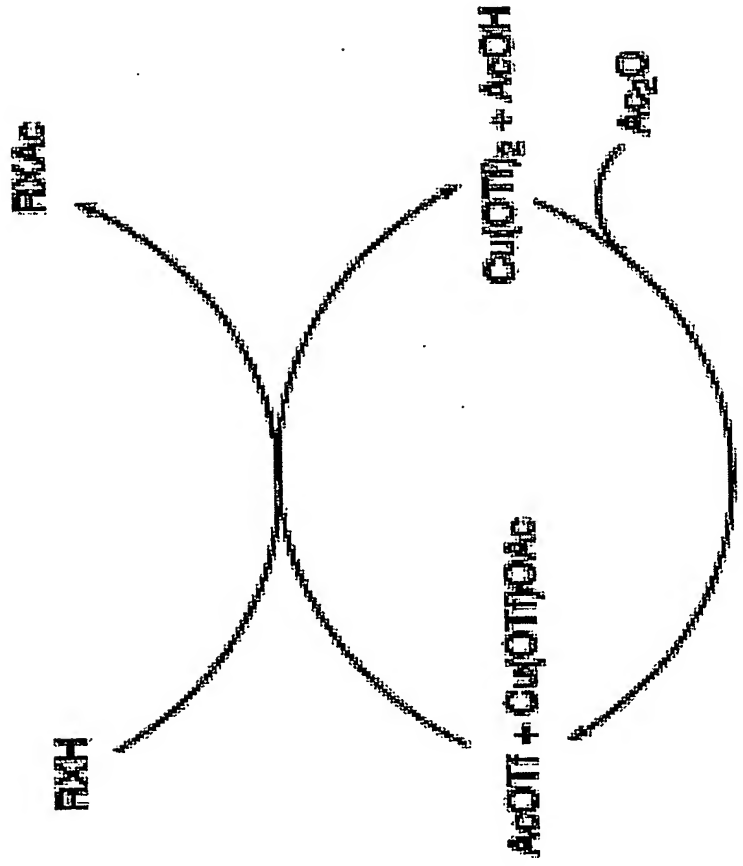
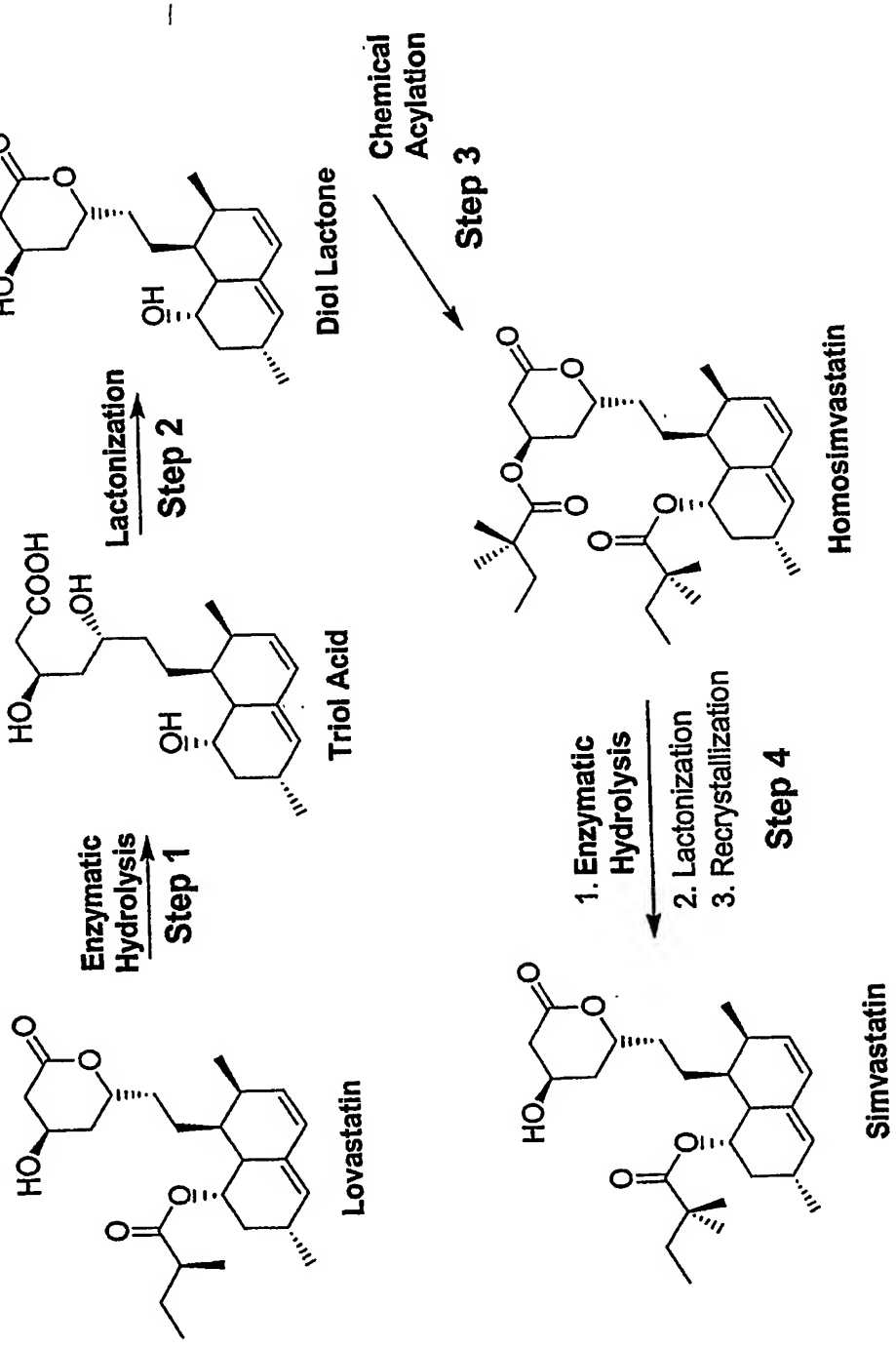


Figure 37

Figure 38


Lovastatin
Enzymatic Hydrolysis
Step 1
Triol Acid
Lactonization
Step 2
Diol Lactone
Chemical Acylation
Step 3
Homosimvastatin
1. Enzymatic Hydrolysis
2. Lactonization
3. Recrystallization
Step 4
Simvastatin

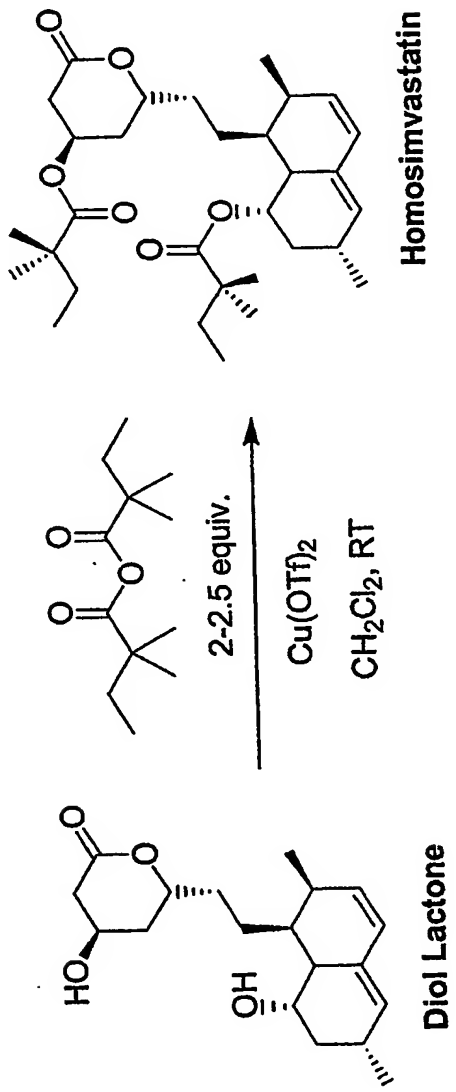

Homosimvastatin
2-2.5 equiv.
Cu(OTf)2
CH2Cl2, RT
Diol Lactone
HO
OH

Figure 39

Figure 40

Figure 40A

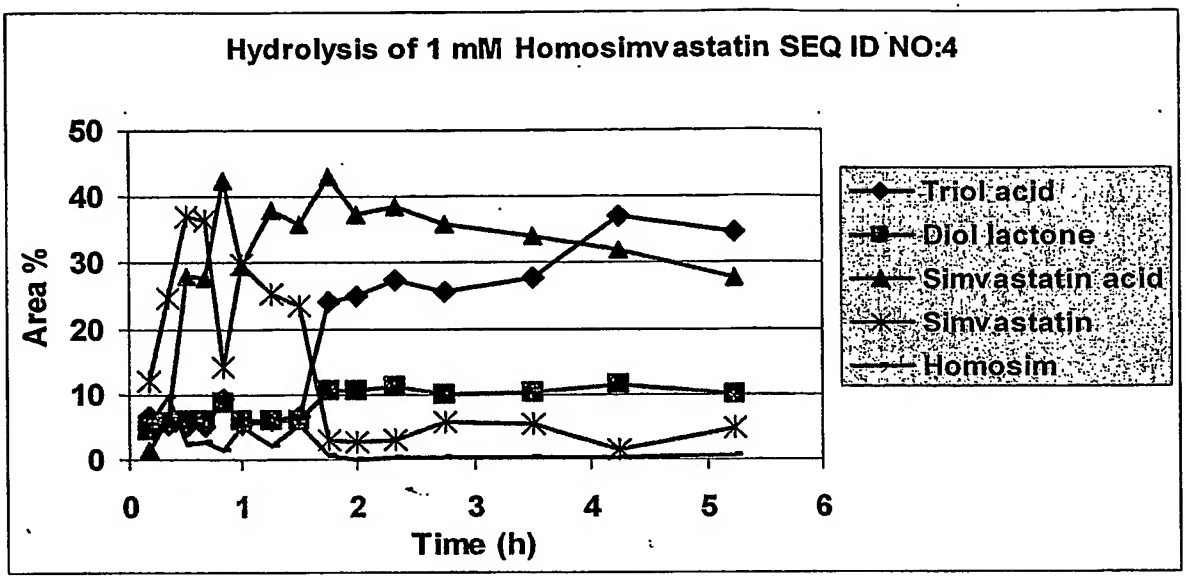


Figure 40B